Rev Iberoam Micol 1999; 16: 77-81 

# Wood, animals and human beings as reservoirs for human *Cryptococcus neoformans* infection

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*Cryptococcus neoformans* was first cultivated by Sanfelice from fermenting peach juice in 1894. At the same time, Busse and Buschke reported, separately, the first case of human disease caused by this yeast. During the next years, the fungus was isolated only from lesions or secretions of men or animals. In 1951, Emmons reported the isolation of *C. neoformans* from soils of Virginia and, in 1955, demonstrated that virulent strains of *C. neoformans* were found commonly and abundantly in pigeon manure under roosting sites. Since cryptococcosis is not contagious, and virulent strains of *C. neoformans* are not isolated from skin, mucosa or feces of man with sufficient frequency to support the concept of an endogenous source of infection, Emmons concluded that the exposure of men and animals to pigeon excreta could explain adequately the epidemiology of cryptococcosis [1]. This saprophytic source of *C. neoformans* has been recognized in many areas of the world, but many questions about the ecology of *C. neoformans* and the epidemiology of cryptococcosis still remain unanswered.

*C. neoformans*, the anamorph of *Filobasidiella neoformans*, is an encapsulated basidiomycetous yeast with two varieties - var. *neoformans* (serotypes A, D, and AD) and var. *gattii* (serotypes B and C) - with distinct life cycles, physiology, ecology and genetics [2,3]. Infection caused by *C. neoformans* var. *gattii* is restricted to some geographical areas, mainly tropical and subtropical regions, occurring predominantly in non-immunocompromised individuals [4,5], whereas reported cases of opportunistic cryptococcosis throughout the world are usually due to *C. neoformans* var. *neoformans*. In nature, *C. neoformans* var. *gattii* has been isolated from plant debris of *Eucalyptus* trees in some endemic areas of cryptococcosis due to the variety *gattii* [6], but it has not been demonstrated in avian droppings and soils contaminated by avian excreta, the major saprophytic source of *C. neoformans* var. *neoformans*. The ecology of *C. neoformans* was recently reviewed by Sorrell and Ellis [7].

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Apdo. 699, E-48080 Bilbao (Spain).
1130-1406/99/5.00 Euros

Human infection by *C. neoformans* is thought to be acquired by inhalation of airborne propagules from an environmental source [3]. However, evidences for an epidemiological association between exposure to saprophytic sources of *C. neoformans* and human infection are circumstantial. *C. neoformans* yeast cells in soils and in avian nesting areas possess minimal capsule, may be smaller than 2 µm, and are easily aerosolized. On the other hand, basidiospores of *F. neoformans*, which have been demonstrated to be pathogenic in animal experiments, have characteristics that favor them to be more readily deposited in the lungs than the yeast cells: they are smaller (1.8 x 2.5 µm in diameter), easily aerosolized, and much more resistant to desiccation than yeast cells [8].

## Human infection and exposure to natural sources of *C. neoformans* var. *neoformans*

Avian droppings are considered a selective natural substratum for *C. neoformans* [9]. Pigeon and other birds may act as mechanical carriers of *C. neoformans* var. *neoformans* on their beaks, legs, and feathers, but the actual role of avian species in the ecology of the fungus is not fully understood. The source of *C. neoformans* in avian droppings remains obscure. Cryptococcosis has been rarely described in birds [2,10]. When *C. neoformans* is fed to pigeons, it can survive the gastrointestinal passage, but it is found in their droppings only for a short time. The multiplication of the fungus is not supported in the gut of pigeons [11], but the crop seems to be a better place for *C. neoformans* than the rest of the digestive tract, a site where an endosaprophytic phase of *C. neoformans* var. *neoformans* could occur [11].

*C. neoformans* var. *neoformans* is isolated from droppings of a large variety of avian species other than pigeon, mainly Psittacidae birds [12,13]. It is possible that some birds offer better conditions for the survival and multiplication of *C. neoformans* var. *neoformans* in their excreta than others [14] as well as different feeding habits may directly expose them to infectious propagules of the fungus from other environmental sources, such as wood [15,16].

The primary natural habitat of *C. neoformans* could be plant species and decaying wood, places where the natural occurrence of the sexual state of *C. neoformans* is also able to develop [2,17]. In laboratory conditions, hay extracts as well as dead and decaying plants constitute suitable substrata for the growth of *C. neoformans* [9]. In Rio de Janeiro, *C. neoformans* var. *neoformans* was repeatedly isolated from decaying wood present on the inner surface of hollows occurring in the trunks of

several different trees [16,18]. Lazera *et al.* [16] suggest that *C. neoformans* var. *neoformans* seems not to be associated with a particular tree but rather with a specialized niche resulting from the natural biodegradation of wood. In decaying wood, xylose, cellobiose and other byproducts of the degradation of lignin and xylan might support saprophytic growth of *C. neoformans* [16,19]. *C. neoformans* has diphenol oxidase, an enzyme identified as part of the enzymatic activity capable of mineralizing lignin [16], which suggests that phenol oxidase activity of *C. neoformans*, recognized as a factor of virulence for animal infection, might be an adaptation to its natural habitat [19]. Decaying wood could also be the principal source of *C. neoformans* var. *gattii* in nature [19].

Human exposure to *C. neoformans* var. *neoformans* seems to be common and can occur in a number of places from where the fungus has been isolated, like old buildings, churches, squares, zoological gardens, barns, stables, and even domiciles [13,20]. Primary cryptococcal infection in humans is often subclinical, but its frequency has been impossible to estimate, since a satisfactory skin test antigen has not yet been developed for population surveys. Serological testing has revealed unusually high rates of infection among pigeon breeders, but no greater rate of disease, indicating that contact with pigeon and their excreta results in more frequent exposure to *C. neoformans* and/or its antigens [21].

Although the precise mechanisms of effective resistance to *C. neoformans* have not been established, an intact cell-mediate immunity seems to be crucial for adequate resistance to cryptococcosis caused by the variety *neoformans* [21]. Incidence of opportunistic cryptococcosis has increased markedly in recent years due to its frequent occurrence in patients with AIDS. Almost all cases of cryptococcosis in AIDS patients are due to the variety *neoformans*, regardless of geographical location [3]. The risk of cryptococcal meningitis increases with decreasing $CD4^+$ lymphocyte counts in HIV-infected individuals [22]. For instance, cryptococcosis was recently reported in an AIDS patient with a $CD4^+$ lymphocyte count of 50/mL who had been exposed to *C. neoformans* var. *neoformans* while cleaning a newly rented flat where pigeon manure had accumulated. Three HIV-positive friends living in the flat, all of them with a $CD4^+$ lymphocyte count higher than 150/mL, had been helping with the cleaning but did not have cryptococcosis [23].

Other factors that might determine the pathogenesis of cryptococcosis, like virulence of the strain and size of the inoculum, are not clearly established [21]. Cryptococcosis has been reported in both previously healthy persons and immunocompromised individuals exposed to materials heavily contaminated with *C. neoformans* var. *neoformans* [3,22,23]. Investigations carried out in Central Africa by Swinne *et al.* showed that AIDS patients with cryptococcosis are frequently exposed to *C. neoformans* var. *neoformans* in their immediate daily, domestic environment [20]. In Rio de Janeiro, AIDS patients exposed to *C. neoformans* var. *neoformans* in the domiciliar environment had a risk for cryptococcosis twice higher than AIDS patients not exposed to the fungus in their domiciles (Odds ratio=2.05) [13]. Attempts to isolate *C. neoformans* from domiciliar environment in Barcelona have been, however, unsuccessful [24].

Since exposure to *C. neoformans* var. *neoformans* seems not to be uniform from place to place, the frequency and intensity of human exposure to environmental sources of *C. neoformans* var. *neoformans* might account for differences in the prevalence of cryptococcosis among AIDS patients in distinct geographical areas: from 2% to 10% in Western Europe and United States to over 15% in some countries in Africa [22].

Several typing methods have been used to characterize *C. neoformans* molecularly. These studies demonstrate genetically distinct populations of *C. neoformans* in different parts of the world as well as within a same location. Attempts have been made to characterize and compare clinical and environmental isolates of *C. neoformans* var. *neoformans* from geographically defined regions.

In New York City, Currie *et al.* [25], using restriction fragment length polymorphism (RFLP), observed that some strain types were present at multiple environmental sites (pigeon habitats) separated by up to 9 Km, indicating a wide distribution of those strains within this area. On the other hand, genetically different strains were recovered from sites less than 0.5 Km apart and even from the same pigeon excreta sample. Two strain types, isolated from one patient each, were also isolated from environmental sites, but not from areas of the patients' addresses. In Nagasaki, genetic diversity among clinical isolates of *C. neoformans* var. *neoformans* was not demonstrated by RFLP, but a random amplified polymorphic DNA (RAPD) analysis using an arbitrary primer (AP)-PCR method allowed to discriminate and compare clinical and environmental isolates [26]. In that study, some isolates from pigeon habitats were strongly associated with those from patients. In Bordeaux, an area where cryptococcosis due to *C. neoformans* var. *neoformans* serotype D is frequently reported, pigeon droppings were demonstrated to contain a genetically heterogeneous population of *C. neoformans* serotypes A and D in which some isolates were genetically similar to clinical isolates [27]. In Central Africa, RFLP analysis showed that three clinical isolates of *C. neoformans* var. *neoformans* (two from Burundi and one from Zaire) had identical pattern, but this pattern was not found in the isolates which were recovered from the dust in and around the patients' homes [28].

Reports that seek to determine the relationship between cryptococcosis and exposure to saprophytic sources have been restricted by poor understanding about fungal infection and disease. *C. neoformans* var. *neoformans* is ubiquitous, which means that the area selected for sampling is not necessarily the area where the patient acquired his/her infection. The time interval between inhalation of *C. neoformans* and expression of disease is not known and some cases can arise from reactivation of a latent focus rather than from exposure to a saprophytic source of the fungus. When clinical infection is the result of reactivation of latent disease, temporal association between exposure to environmental sources of *C. neoformans* and disease onset may be obscured [13,25].

Some genetic types of *C. neoformans* found in patients but not found among environmental isolates might reflect insufficient environmental sampling or acquisition of infection elsewhere [25,28]. On the other hand, the finding that some patients are infected by strains of *C. neoformans* var. *neoformans* which are genetically related to those found in pigeon excreta supports pigeon excreta as a reservoir for pathogenic *C. neoformans* var. *neoformans*, but does not prove that patients were infected by exposure to pigeon excreta [25,26]. Moreover, some isolates of *C. neoformans* var. *neoformans* serotype A from distinct geographical areas show genetic concordance, suggesting a clonal population structure for *C. neoformans* rather than a recombining population [29]. Considering clonality in *C. neoformans* var. *neoformans* population, certain clones could be globally dispersed by wind transport and/or bird migrations [29].

It is not known whether reinfection by *C. neoformans* occurs. DNA typing analysis of initial and relapse isolates of *C. neoformans* var. *neoformans* from patients with recurrent cryptococcal meningitis has revealed persistence of the initial strain rather than reinfection with newly acquired strains [30]. However, involvement of multiple *C. neoformans* strains in a single episode of cryptococcosis as well as reinfection with a novel strain in recurrent infection were demonstrated by RAPD and DNA fingerprinting [31].

The association between cryptococcosis and exposure to other saprophytic sources of *C. neoformans* var. *neoformans*, like plant debris and hollows of living trees, has not been investigated.

## Human infection and exposure to natural sources of *C. neoformans* var. *gattii*

In 1990, Ellis and Pfeiffer reported the isolation of *C. neoformans* var. *gattii*, serotype B, from plant debris (wood, bark, leaves, flowers) collected under the canopies of flowering *Eucalyptus camaldulensis* (red river gum trees) growing in an Australian endemic area of cryptococcosis due to the variety *gattii* [32]. Thereafter, *C. neoformans* var. *gattii* was isolated from *E. camaldulensis* in California, USA, and from plant debris of *E. tereticornis* (forest red gum), a species closely related to *E. camaldulensis*, in Australia [33]. These findings suggested a specific association between *C. neoformans* var. *gattii* and those trees. Since both species of *Eucalyptus* have been exported extensively from Australia to other regions from where cryptococcosis caused by the variety *gattii* is reported, Pfeiffer and Ellis hypothesized that *C. neoformans* var. *gattii* has been exported from Australia to other regions by infected seeds and seedlings [19].

*C. neoformans* var. *gattii*, serotype B, was isolated from *Eucalyptus camaldulensis* in Apulia, Italy [34], in the northern state of Punjab, India [35], and in the state of Piaui, northeastern Brazil [36]. In Mexico, it was isolated from *E. tereticornis* [37]. In the San Diego Zoo area, it was isolated from two *Eucalyptus* spp., one of which was further identified as *E. citriodora* [38]. Recently, the variety *gattii* was demonstrated in wood debris from both *E. rudis* (flooded gum), a member of the red gum group, and *E. gomphocephala* (tuart), a species with no known close relatives, growing in Australia [6].

In Australia, exposure to *E. camaldulensis* trees provides a plausible explanation for the high incidence of infections caused by the variety *gattii* in persons living in close association with eucalyptus trees in endemic areas of *C. neoformans* var. *gattii* serotype B [32]. Genetic concordance between the majority of the Australian clinical isolates of *C. neoformans* var. *gattii* and environmental isolates recovered from eucalyptus material substantiates this hypothesis [38]. All Australian eucalyptus isolates of *C. neoformans* var. *gattii* are serotype B and RAPD profile VGI, independent of the *Eucalyptus* species [6]. Most clinical isolates are also serotype B and RAPD profile VGI, but various clinical isolates assigned to profile VGII have been recently recognized in some areas of Australia [39,40], suggesting that an yet unknown environmental source of *C. neoformans* var. *gattii* profile VGII seems to exist in these areas.

In Papua New Guinea, the distribution of disease due to *C. neoformans* var. *gattii* does not mirror the distribution of imported or endemic *Eucalyptus* species and attempts to isolate *C. neoformans* from 1130 samples of dust, soil, and vegetation, including *Eucalyptus* and other species of trees, collected in and around the patients' houses, were unsuccessful [41]. In Central Africa, examination of 657 *Eucalyptus* specimens collected in Rwanda did not detect *C. neoformans* in any type of plant material [42]. In an urban area in northeastern Brazil, where cryptococcosis caused by the variety *gattii* is endemic, *C. neoformans* var. *gattii* serotype B was repeatedly isolated from the hollow of a pottery tree (*Moquilea tomentosa*) in a site where no *Eucalyptus* trees were observed [43]. In an area of native rain forest from Brazilian Amazon Region, *C. neoformans* var. *gattii*, serotype B, was isolated from decaying wood of a tropical tree [44], further identified as *Guettarda* sp.

Although most reported cases of cryptococcosis due to *C. neoformans* var. *gattii* serotype C occur in California, all environmental isolates of *C. neoformans* var. *gattii* from this area are identified as serotype B [2]. The first environmental isolate of *C. neoformans* var. *gattii* serotype C was recently reported on sandy-nature detritus collected from two of 68 almond trees (*Terminalia catappa*) in the city area of Cúcuta, Colombia, a region in the northeast of the country recognized as an endemic area of cryptococcosis due to the variety *gattii*, serotype B [45].

The isolation of *C. neoformans* var. *gattii* from trees other than *Eucalyptus* suggests that different plant species could be reservoirs for *C. neoformans* var. *gattii* in distinct geographical areas, reinforcing that other sources of *C. neoformans* var. *gattii* should be sought and the linkage with human disease verified. Cryptococcosis due to *C. neoformans* var. *gattii* was diagnosed in a German patient who had never left his country but worked in sawmills and woodworking factories. The possible source of infection was thought to be dust of tropical woods, but attempts to isolate the fungus from 477 samples of tropical wood were unsuccessful [15]. Autochthonous cryptococcosis due to the variety *gattii* was recently reported in goats in Cáceres, Spain [46]. In France, three out of 413 clinical isolates of *C. neoformans* were identified as variety *gattii* serotype B. One isolate was from a Cambojan man, whose disease could be due to endogenous reactivation of latent infection, but the other two patients had not traveled outside the country, one of whom sold exotic fruits and the other one owned a bird imported from Central Africa [47].

Disseminated cryptococcosis caused by *C. neoformans* var. *gattii* was recently reported in New Zealand in a North Island brown kiwi (*Apteryx australis mantelli*), a flightless nocturnal bird which has a body temperature lower than other avian species [10]. An isolate of *C. neoformans* var. *gattii* recovered from the nasal cavity of an African grey parrot was included in a genetic study of animal isolates in Australia [39]. However, since *C. neoformans* var. *gattii* has thermotolerance levels lower than the variety *neoformans*, birds seem to be a less probable vector of the variety *gattii* than hosts with a body temperature not exceeding 35 to 37∞C, such as insects, bats, koalas, and other mammals [18,48]. *C. neoformans* var. *gattii* was isolated from a nest of the wasp *Polybia occidentalis* in Uruguay, from bat guano in Rio de Janeiro [18], from feces and paws of koalas in Australia [48], and also from camel hair and ostrich feathers in a wildlife park in Apulia [34].

Human cryptococcosis due to the variety *gattii* occurs predominantly in non-immunocompromised individuals. The apparent rarity of *C. neoformans* var. *gattii* infections in AIDS patients is an unexplained observation. Considering that most HIV-infected individuals reside in urban areas, they might be less exposed to environmental sources of *C. neoformans* var. *gattii* than to sources of the

variety *neoformans* [15,48]. Differences in the pathogenesis of the infections caused by the two varieties of *C. neoformans* and host-fungus interactions might justify the preponderance of infection caused by the variety *neoformans* in HIV-infected individuals, since the lack of exposure to *C. neoformans* var. *gattii* alone seems to be insufficient to explain differences in areas where cryptococcosis due to the variety *gattii* is endemic [4,49]. In Australia, for instance, cryptococcosis due to *C. neoformans* var. *gattii* was described in cats that had not ventured outside the greater metropolitan area of Sydney [39].

## Is there a human reservoir for *C. neoformans*?

Reservoir host is defined as "an alternate or passive host or carrier that harbors pathogenic organisms, without injury to itself, and serves as a source from which other individuals can be infected" [50].

*C. neoformans* can be isolated from oropharynx, nares, skin and sputum of human beings without causing a pathogenic condition, defining a colonization of skin and mucosa [51-55]. The upper respiratory tract and nasal cavities could be a site from where *C. neoformans* infection spread, though an animal experiment suggests that the olfactory mucosa might protect from infection by *C. neoformans* without the intervention of the immune system cells [56].

Presence of *C. neoformans* in sputum can be transient or occur over a lengthy period. Lung diseases like chronic bronchitis, tuberculosis, and cancer are conditions that favor cryptococcal colonization, but do not predispose to pulmonary or disseminated cryptococcosis [52]. In sputum cultures the number of *C. neoformans* colonies is frequently low [53], but Randhawa and Paliwal [55] reported that the cultivation of two loopfuls of sputum from a patient with pulmonary tuberculosis revealed 50 colonies of *C. neoformans*, a number that subsequently rose to nearly 300 and, thereafter, declined until the sputum cultures became negative without any treatment of the patient. It is not clear whether the presence of *C. neoformans* in sputum is, in some cases, an evidence of minimal pulmonary lesion more than a commensal association, but a benign association between *C. neoformans* and human beings seems to exist. Isolation of *C. neoformans* from the sputum of patients over a long time reinforces this hypothesis [51] as well as suggests that sputum and upper respiratory tract mucosa might provide a good substratum for the survival and multiplication of the fungus.

Persistence of *C. neoformans* in the prostate of patients adequately treated for cryptococcal meningitis also suggests that the urinary tract is a sequestered reservoir of infection from which systemic relapse may occur [57].

Since *C. neoformans* is able to colonize sputum and nares of men and animals, it is conceivable that individuals could acquire cryptococcosis from the infected discharge of human beings as well as from animals. However, cryptococcosis is classically described as a non-contagious disease. There are no evidences of human-to-human transmission, except for a reported case of panophthalmitis after a corneal transplant and another one of cutaneous cryptococcosis after accidental intracutaneous inoculation of blood from a patient with AIDS and cryptococcemia [3]. Animal-to-human transmission has also never been proven, even though both varieties of *C. neoformans* have been isolated from nares and upper respiratory tract of cats and dogs with or without cryptococcosis [39,58,59]. Human beings and animals are not a known source of infection probably because the yeast cell diameter in tissues and body fluids varies in size from 5 to 10 µm in diameter, with a capsule that also varies considerably in thickness, from a few micrometers to a width that equals or exceeds the diameter of the cell [21]. This size is not optimal for lung deposition. Most strains of *C. neoformans*, even those consistently small-capsuled in vitro, develop large capsules during infection and capsule-free isolates have been rarely found in tissue [21].

These findings could explain the non-occurrence of person-to-person transmission, but human beings and other mammals could spread the fungus to the environment. However, *C. neoformans* has not been isolated from air or dust collected from wards occupied by patients with cryptococcosis [20]. In Central Africa, the DNA fingerprinting pattern observed in three clinical isolates of *C. neoformans* var. *neoformans* was not found in the isolates recovered from indoor dust [28]. In Rio de Janeiro, the frequency of isolation of *C. neoformans* var. *neoformans* from dwellings of AIDS-associated cryptococcosis patients was similar to that observed in domiciles of apparently healthy persons [13].

Even considering that these findings do not support the hypothesis that patients presenting cryptococcosis could contaminate their own environment with the fungus, the possibility should not be completely disregarded. In Australia, the single environmental isolate of *C. neoformans* var. *gattii* RAPD profile VGII was obtained from plant debris along the fence line of a paddock containing sheep infected with *C. neoformans* var. *gattii*, RAPD profile VGII. It is uncertain whether the plant material was contaminated with *C. neoformans* var. *gattii* from the respiratory secretions of the sheep or represented an environmental niche of the VGII strain [39]. It is interesting to note that Cobcroft *et al.* reported the isolation of *C. neoformans* from a bagpipe used by a patient with cryptococcosis: since sputum's patient culture grew *C. neoformans*, it is possible to question whether the patient was infected from the bagpipe or the patient was the source of the bagpipe colonization by the fungus [60].

Many new facts have been collected about the ecology of *C. neoformans* and epidemiology of the cryptococcosis, but much work remains to be done in order to improve our understanding about the peculiarities of *C. neoformans*.

*The author is grateful to Dra. Jacqueline Anita de Menezes, from the Hospital dos Servidores do Estado, Dra. Márcia dos Santos Lazéra and Dr. Bodo Wanke, from the Laboratório de Micologia Médica, FIOCRUZ, for their comments.*

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